Effect of Tyrosine Ingestion on Cognitive and Physical Performance Utilising an Intermittent Soccer Performance Test (iSPT) in a Warm Environment

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Abstract

Purpose: The aim of this study was to investigate the effect of tyrosine ingestion on cognitive and physical performance during soccer-specific exercise in a warm environment. **Methods:** Eight male soccer players completed an individualised 90-minute soccer-simulation (iSPT), on a non-motorised treadmill, on two occasions, within an environmental chamber (25°C, 40% RH). Participants ingested tyrosine (TYR; 250 mL sugar free drink plus 150 mg·kg body mass^{-1} TYR) at both 5h and 1h pre-exercise or a placebo control (PLA; 250 mL sugar free drink only) in a double-blind, randomised, crossover design. Cognitive performance (vigilance and dual-task) and perceived readiness to invest physical effort (RTIPE) and mental effort (RTIME) were assessed: pre-exercise, half-time, end of half-time and immediately post-exercise. Physical performance was assessed using the total distance covered in both halves of iSPT. **Results:** Positive vigilance responses (HIT) were significantly higher (12.6 ± 1.7 v 11.5 ± 2.4, $p = 0.015$) with negative responses (MISS) significantly lower (2.4 ± 1.8 v 3.5 ± 2.4, $p = 0.013$) in TYR compared to PLA. RTIME scores were significantly higher in the TYR trial when compared to PLA (6.7 ± 1.2 v 5.9 ± 1.2, $p = 0.039$). TYR had no significant ($p > 0.05$) influence on any other cognitive or physical performance measure. **Conclusion:** The results show that TYR ingestion is associated with improved vigilance and RTIME when exposed to individualised soccer-specific exercise (iSPT) in a warm environment. This suggests that increasing the availability of TYR may improve cognitive function during exposure to exercise-heat stress.

Keywords: Central fatigue; Tyrosine; Cognitive function; Intermittent exercise; Heat.

Abbreviations:

5-HT Serotonin
CNS Central nervous system
DA Dopamine
HR Heart rate
iSPT Intermittent soccer performance test
LNAA Large neutral amino acids
NA Noradrenaline
NMT Non-motorised treadmill
PLA Placebo
RH Relative humidity
RPE Rating of perceived exertion
RTIME/RTIPE Readiness to invest mental/physical effort
TSS Thermal sensation
TYR Tyrosine

Introduction

Soccer is the most widely played team sport in the world and is characterised as high-intensity, intermittent exercise, performed over a 90 minute period (Stølen et al. 2005). Successful performance in soccer is dependent upon the simultaneous execution of technical, physical and mental skills (Meeusen et al. 2006a). However, the demanding, intermittent nature of the sport places players under high physiological strain and as a consequence, the ability to perform high-intensity exercise and maintain cognitive function declines towards the end of a match, due to the development of fatigue (defined as the inability to maintain work at a given intensity) (Bangsbo et al. 2006). The outcome of the game is highly dependent upon the ability of the players to cope with this fatigue and maintain physical and cognitive performance (Özgünen et al. 2010). This is reinforced by statistics from the European Soccer Championships (2004) demonstrating that a significantly higher percentage of goals were scored in the later stages of the second half (57.4%) compared to the first half (42.6%) (Yiannakos and Armatas 2006), attributed to lapses in concentration and mental fatigue in the opposing team (Reilly 1997).

Competitive soccer is often played in hot environments by recreational and elite players alike (Özgünen et al. 2010), imposing an additional stress on the body (exercise-heat stress). This added stress can accelerate the onset of fatigue (Mohr et al. 2012), progressively impairing exercise performance (Gonzalez-Alonso et al. 1999; Nybo et al. 2014) and cognitive function (Maughan et al. 2007; Simmons et al. 2008; Gaoua et al. 2011). Previous research has focused on peripheral mechanisms of fatigue, suggesting endogenous substrate depletion is the primary cause (Galloway and Maughan 1997; Bangsbo et al. 2006), however it is now clear that there is also a significant involvement of the central nervous system (CNS) and psychological factors (Nybo et al. 2014). This shows that fatigue is a complex phenomenon, occurring at all levels of the brain-muscle pathway (Roelands and Meeusen 2010).

There are several theories of central fatigue (Cheung and Sleivert 2004), however the original central fatigue hypothesis is based on the concept that during prolonged exercise, the activity and synthesis of the central monoamines are altered, specifically serotonin (5-HT), dopamine (DA) and noradrenaline (NA) (Newsholme 1987; Meeusen et al. 2006b). An increased ratio of brain DA:5-HT is suggested to

augment performance during prolonged exercise while low ratios induce lethargy and losses in motivation (Davis and Bailey 1997). Therefore, DA and NA are considered important neurotransmitters involved in both physical and cognitive performance due to their direct association with changes in arousal, motivation and motor control (McMorris et al. 2006; Watson 2008). Conversely, opposing evidence suggests that an increase in central NA decreases performance in the heat, as demonstrated by Roelands et al. (2008). During exercise, there is an elevation in concentrations of central catecholamine neurotransmitters in several cerebral regions, observed in the striatum and hypothalamus of rodents (Meeusen et al. 1997; Foley and Fleshner 2008). However, at the point of exhaustion, brain-tissue DA content (in rodents) is markedly decreased (Bailey et al. 1993), suggesting that the DA availability may be a possible mechanism for exercise induced fatigue (Watson et al. 2012). This knowledge proffers the opportunity to manipulate the CNS with nutritional and pharmacological intervention strategies to attenuate the onset of fatigue during soccer match-play.

Many nutritional manipulation strategies are utilised in soccer (Nedelec et al. 2013) as small dietary mediated improvements in performance could significantly alter game outcome, by providing players with an advantage over their opponents. The precursor for catecholamine synthesis is tyrosine (TYR), a non-essential amino acid found in protein rich dietary sources and synthesised in the liver from phenylalanine (Wurtman et al. 1980). Supplementation of TYR increases its ratio to other large neutral amino acids (LNAA) for competitive transport across the blood-brain-barrier, thus resulting in a greater cerebral uptake and an increase in DA and NA synthesis (Fernstrom and Faller 1978; Gibson and Wurtman 1978). Previous research involving TYR (100-300 mg·kg body mass^{-1}) is primarily military based, finding improvements in certain aspects of cognitive function after exposure to stressful environments such as cold (Banderet and Lieberman 1989; Mahoney et al. 2007; O'Brien et al. 2007) and hypoxia (Banderet and Lieberman 1989), and paradigms involving both extended wakefulness (Neri et al. 1995) and the physical/emotional stress nexus (Deijen et al. 1999). Specific to hot environments, Tumilty et al. (2011) demonstrated a 15 ± 11% increase in exercise capacity during constant-load, continuous cycling in the heat (30°C; 50% RH) after TYR ingestion (150 mg·kg body mass^{-1}). However, to date, this is the first and only study to observe a beneficial effect of TYR on physical performance (with or without heat stress) in humans. More recently, similar studies have failed to replicate this finding during exercise to exhaustion (Watson et al. 2012) and a self-paced time

trial (Tumilty et al. 2014) in the heat, despite the same dosage strategy and comparable increase in circulating TYR.

It appears that supplementing with TYR may alleviate stress-related decrements in cognitive function and possibly offset the perception of fatigue during exposure to demanding environments. It is yet to be elucidated whether TYR has a positive effect on physical and cognitive performance aspects during soccer-specific exercise. Therefore, the aim of the present study was to investigate the effect of acute TYR ingestion on both cognitive and physical performance utilising an individualised, valid and reliable non-motorised treadmill (NMT) based soccer simulation (iSPT) (Aldous et al. 2013), in a warm environment (25 °C). It was hypothesised that a dose of 300 mg·kg body mass^{-1} TYR would improve cognitive performance and increase the distance covered during iSPT, when compared to placebo.

Methods

Subjects

Eight healthy, University level soccer players (mean age 21 ± 1 years, height 180.3 ± 6.2 cm, body mass 74.9 ± 8.7 kg, body fat percentage 11 ± 5 % and physical activity 6.3 ± 1.2 h·wk^{-1}), volunteered to participate in this study. Prior to participation, subjects received detailed information about the study and subsequently provided their written informed consent. Subjects were not acclimated to exercising in the heat and had never consumed a supplementary dose of TYR before this study. Ethical approval was gained from the University of Bedfordshire Research Ethics Committee.

Familiarisation

Subjects were required to attend 3 familiarisation sessions prior to the experimental trials, involving shortened bouts of the soccer-simulation protocol (iSPT) (Aldous et al. 2013) on a NMT (Woodway, Force 3.0, Cranlea, Birmingham) in temperate conditions (18 °C). The activity pattern of the iSPT protocol is based on previous soccer match-play data and involves several movement categories (stand, walk, jog, run, fast run, variable run and sprint) (Aldous et al. 2013). Rigorous familiarisation [described in full in Aldous et al. (2013)] to iSPT ensured movement categories were individualised to each participants sprint speed determined from a peak speed assessment on the NMT. Additionally,

subjects were familiarised to the visual and audio cues presented to them by a computer program (Innervation, Pacer Performance System Software), which displayed their actual speed (red line) and a target speed (green line) that they were instructed to follow as closely as possible. Within these sessions, subjects also performed two demonstration versions of the vigilance and dual-task cognitive PsychE software tests (Hope et al. 1998). The familiarisation sessions were employed to ensure that subjects were accustomed to the protocol and were deemed appropriate to minimize any learning effects of the cognitive assessments (Hope et al. 1998) and iSPT (Aldous et al. 2013). Once familiarised with the protocol, subjects returned to the laboratory in a fasted state to have their body fat percentage assessed utilising bioelectrical impedance (Body Composition, Tanita, BC41MA Segmental Body, Cranlea).

Experimental Procedure

During the experimental trials, each subject attended the laboratory on two separate occasions with at least 7 days between visits. Subjects refrained from alcohol, caffeine and unaccustomed exercise 24 h prior to the testing and completed food diaries to ensure replication of food intake prior to each performance of iSPT, in line with previous research in this area (Chinevere et al. 2002; Tumilty et al. 2011; Watson et al. 2012). Experimental controls were monitored via a questionnaire, with adherence confirmed at 100 % in all instances.

Upon arrival at the laboratory between 0700 and 0900, subjects orally ingested the first dose of either placebo [PLA (250 mL sugar free lemon squash) (Tesco, Bedford, UK)] or tyrosine [TYR (same as PLA plus 150 mg·kg body mass^{-1} TYR powder) (Myprotein.co.uk)]. After a 4 h rest period subjects ingested an identical second dose (300 mg·kg body mass^{-1} TYR in total) between 1100 and 1300 and then rested for 1 h prior to the start of the protocol. The drinks were prepared and coded by a separate laboratory technician to ensure that they were administered in a double-blind, randomised fashion. The drinks were provided in opaque sports bottles and were indistinguishable in taste and texture to the subjects. Prior pilot work confirmed that the dose of TYR administered in this study did not induce any side effects and this administration strategy has previously been shown to alleviate cold-induced decrements in psychomotor performance (O'Brien et al. 2007) and working memory (Mahoney et al. 2007). The TYR supplement used in the present study was analysed via high-performance liquid chromatography (HPLC) to assess its purity [using the method described by Watson et al. (2012)], and

was found to contain a high concentration of TYR (>90%).

Prior to exercise, nude body mass (Scales, Tanita, BWBO800, Allied Weighing) and height (Stadiometre, Harpenden, HAR-98-602, Holtain) were recorded and a urine sample was provided by the subject to assess hydration status using a urine refractometer (Atago Vitech scientific, Pocket PAL-OSMO, HaB Direct). Subjects were instructed to drink 500 mL of water 2 h prior to exercise in line with Sawka et al. (2007) and were deemed euhydrated if urine osmolality was <600 $mOsm \cdot Kg^{-1}$ H_2O (Hillman et al. 2011; Hillman et al. 2013). This experimental control was not breached prior to any experimental procedure commencing. A heart rate monitor (Polar, FS1, Cranlea) was attached and a rectal thermometer (Henleys, 400H & 4491H) inserted 10 cm past the anal sphincter. Skin temperature probes (Grant, EUS-U-VS5-0, Wessex Power) were attached to four skin sites: upper arm, chest, thigh and lower leg, using adhesive tape (Ramanathan 1964). Specific data loggers were used to record rectal (Libra Medical, ET402, Cranlea) and skin (Grant, Squirrel Series, model 451, Wessex Power) temperature. Subjects then entered the custom built Environmental Chamber set at 25 °C and 40 % RH, where they completed the cognitive assessments (vigilance and dual task) at rest.

The vigilance tests (2 min in duration) involved a number display where three digit numbers flashed up on a laptop screen at a rate of 100 per min with an 8% duplication rate. Subjects pressed the spacebar when a duplicated number appeared twice in a row and were scored on the amount of HIT (correct response), MISS (missed cue) and FALSE (false response) scores they achieved. The dual-task tests (3 min duration) required subjects to track a moving target with the mouse cursor and simultaneously respond to random stimuli with the spacebar. The percentages of time on the target (TRACKING) and stimuli responses (MISS and FALSE) were recorded. On completion of both tests, a report was provided, detailing the subject's scores for each test. All cognitive tasks were computer based and delivered in line with previous work in the field (Hope et al. 1998). See Table 1 for further details and description of the vigilance and dual-task assessments.

Insert Table 1 near here please

Measurements

Subsequent to the initial cognitive assessments, subjects rested for 5 min and pre-exercise measures were taken, including heart rate (HR), thermal sensation (TSS) using the 0-8 scale (Young et al. 1987),

rating of perceived exertion (RPE) using the 6-20 Borg scale (Borg 1982), skin temperature (T_{sk}) of all four skin sites, rectal temperature (T_{re}) and readiness to invest physical effort (RTIPE) (Duncan et al. 2012) and mental effort (RTIME) (Duncan et al. 2012) using a 0-10 scale (see Duncan et al. (2012) for specific details of scale).

During iSPT [2 * 45 min halves, interspersed with the half-time period (15 min)], HR, RPE, TSS, T_{re} and T_{sk} were recorded every 5 min and the temperature and humidity of the chamber was recorded continuously. Weighted mean T_{sk} was calculated using the temperatures recorded for all four skin sites, using the following equation ('t' represents temperature):

$$0.3({}^{t}chest + {}^{t}arm) + 0.2({}^{t}thigh + {}^{t}leg)$$ (Ramanathan 1964).

Cognitive function (vigilance and dual-task) and RTIPE and RTIME were assessed at four time points [pre exercise (0 min), onset of half-time (45 min), end of half time (EOHT) and immediately post exercise (90 min)], while subjects were seated in the chamber during the rest periods. Physical performance was assessed using the total distance covered during the first and second half of iSPT in both conditions. All subjects consumed a standardised amount of plain water (250 mL) during the 15 min half-time period and sweat losses were calculated from the difference in pre- and post-exercise body mass, after adjusting for any fluid consumed or urine excreted.

Statistical Analyses

Statistical analyses were completed using IBM SPSS statistics 19.0 (IMB, Corporation, New York). Statistical assumptions were assessed using conventional graphical methods (Grafen et al. 2002) and deemed plausible for each variable. A two-way ANOVA (condition x time) with repeated measures was used to analyse mean differences in cognitive data, distance covered and all physiological, perceptual and thermoregulatory data between conditions (TYR and PLA). Where significance was obtained, Bonferroni *post-hoc* tests were carried out. Assumptions of homogeneity of variance were assessed using Mauchly's test of Sphericity. Dual- task tracking and false scores violated sphericity ($p < 0.05$); therefore a Greenhouse-Geisser correction was applied to the degrees of freedom of the F ratio. Paired samples *t*-tests were performed to analyse the differences in sweat loss and pre-exercise urine osmolality between conditions. Two-tailed statistical significance was accepted at the $p < 0.05$ level. All data are presented as mean ± standard deviation (SD).

Results

Hydration Status

No significant difference was observed in pre-exercise urine osmolality (t_7 = -1.212, p = 0.265) between TYR (128.8 ± 86.8 mOsm·Kg^{-1}) and PLA (172.5 ± 102.2 mOsm·Kg^{-1}). No significant difference was observed in mean sweat loss calculated from pre-post body mass (t_7 = -0.687, p = 0.514) between TYR (1.6 ± 0.6 L) and PLA (1.7 ± 0.6 L).

Heart Rate

A significant effect of time was noted over the 90 min protocol for mean HR ($F_{21,147}$ = 161.387, p < 0.001) with a mean increase of 97 b·min^{-1} and 100 b·min^{-1} from 0 min to 90 min in the TYR and PLA conditions respectively. No significant main effect for condition was observed in mean HR ($F_{1,7}$ = 4.839, p = 0.064) and there was no significant condition x time interaction effect ($F_{21,147}$ = 0.88, p = 0.62) (Fig. 1).

Insert Fig 1 near here please

Temperature Measures

There was a significant effect of time for mean T_{re} ($F_{21,147}$ = 106.941, p < 0.001), with a significant rise in T_{re} throughout both halves and a decrease back to baseline at HT. End T_{re} at 90 min was 38.7 ± 0.4 °C in TYR and 39 ± 0.2 °C in PLA. No significant main effect for condition was observed in mean T_{re} ($F_{1,7}$ = 0.65, p = 0.447) between TYR (38.2 ± 0.3 °C) and PLA (38.3 ± 0.2 °C) and no significant condition x time interaction ($F_{21,147}$ = 1.113, p = 0.341). There was a significant effect of time for mean T_{sk} ($F_{21,147}$ = 21.679, p < 0.001), with an increase in T_{sk} in both halves and a drop back to baseline at HT. No significant main effect for condition was observed in mean T_{sk} ($F_{1,7}$ = 0.009, p = 0.929) between TYR (34 ± 0.8 °C) and PLA (34.1 ± 1.4 °C) and there was no significant condition x time interaction ($F_{21,147}$ = 0.93, p = 0.993) (Fig. 2).

Insert Fig 2 near here please

Subjective Measures

There was a significant effect of time for TSS ($F_{21,147}$ = 61.818, p < 0.001) with an increase throughout exercise reaching end values of 6.8 ± 0.4 in TYR and 7.1 ± 0.4 in PLA, indicating that subjects felt 'very hot' at the 90 min stage. No significant main effect for condition was observed in mean TSS scores ($F_{1,7}$ = 2.154, p = 0.186) between TYR (5.9 ± 0.6) and PLA (6 ± 0.4) and there was no significant condition x time interaction. There was a significant effect of time observed over the 90 min protocol for RPE ($F_{21,147}$ = 96.536, p < 0.001) with an increase throughout exercise. No significant main effect for condition was observed in mean RPE scores ($F_{1,7}$ = 2.299, p = 0.173) between the TYR (13.9 ± 1.3) and PLA (14.2 ± 1.3) and no significant condition x time interaction was noted ($F_{21,147}$ = 0.343, p = 0.997) (Fig. 3).

Insert Fig 3 near here please

Effort Scales

A significant effect of time was noted for RTIPE ($F_{3,21}$ = 31.741, p < 0.001) with a decrease at the end of both halves (45 min and 90 min). No significant main effect was observed for subjects RTIPE scores ($F_{1,7}$ = 0.568, p = 0.476) between the TYR (6 ± 1.7) and PLA (5.6 ± 2.1) conditions and no significant condition x time interaction was observed ($F_{3,21}$ = 2.739, p = 0.069). There was a significant main effect for condition for RTIME scores ($F_{1,7}$ = 6.443, p = 0.039). On average, RTIME was significantly higher by 13 ± 36% in the TYR condition compared to PLA (p = 0.039, 95% CI = 6 to 7). A significant effect of time was noted ($F_{3,21}$ = 28.745, p < 0.001) with a decrease in RTIME at the end of both halves. However, no condition x time interaction was noted ($F_{3,21}$ = 2.75, p = 0.068) (Fig. 4).

Insert Fig 4 near here please

Distance Covered

No significant difference was observed in the distance covered in the first half of iSPT (t_7 = -1.083, p = 0.315) between TYR (4323.6 ± 344.7 m) and PLA (4390.8 ± 241.2 m) or in the second half (t_7 = -0.747, p = 0.497) between the two conditions (4307.6 ± 378.9 m and 4338 ± 322.7 m respectively). Overall the total distance covered was not significantly different (t_7 = -1.025, p = 0.339) between

conditions. There was also no significant difference in distance covered between halves in TYR (t_7 = -0.465, p = 0.656) or PLA (t_7 = 1.176, p = 0.278) (Fig. 5).

Insert Fig 5 near here please

Cognitive Performance

Vigilance

There was a significant main effect for condition for HIT scores ($F_{1,7}$ = 10.17, p = 0.015). On average there was a 9 ± 28% increase in HIT scores in the TYR condition compared to PLA (p = 0.015, 95% CI = 0 to 2). However, there was no significant condition x time interaction ($F_{3,21}$ = 0.06, p = 0.98) or an effect of time ($F_{3,21}$ = 0.14, p = 0.94) on HIT scores. There was a significant main effect for condition for MISS scores ($F_{1,7}$ = 10.95, p = 0.013), with an average decrease of 31 ± 29% in the TYR condition compared to PLA (p = 0.013, 95% CI = 0 to 2). However, there was no significant condition x time interaction ($F_{3,21}$ = 0.05, p = 0.83) or an effect of time ($F_{3,21}$ = 0.2, p = 0.67) on MISS scores. No significant main effect for condition was observed for FALSE scores ($F_{1,7}$ = 0.28, p = 0.61) between the TYR and PLA conditions. Furthermore, there was no significant condition x time interaction ($F_{3,21}$ = 0.77, p = 0.52) or a significant effect of time for FALSE scores ($F_{3,21}$ = 0.12, p = 0.96) (Fig. 6). Table 2 provides HIT, MISS and FALSE values for each time point (0 min, HT, EOHT and 90 min).

Insert Fig 6 and Table 2 near here please

Dual-task

No significant main effect for condition was observed for TRACKING ($F_{1,7}$ = 1.29, p = 0.29). Furthermore, there was no significant condition x time interaction ($F_{1.65,11.51}$ = 0.17, p = 0.8) or effect of time ($F_{2.15,15.1}$ = 1.37, p = 0.29). Similarly there was no significant main effect for condition for MISS scores ($F_{1,7}$ = 0, p = 1.0) and no significant condition x time interaction ($F_{3,21}$ = 0.17, p = 0.8) or effect of time ($F_{3,21}$ = 1.37, p = 0.28). Finally, there was no significant main effect for condition for FALSE scores ($F_{1,7}$ = 0.16, p = 0.70) and no significant condition x time interaction ($F_{1.33,7.95}$ = 0.49, p = 0.56) or effect of time ($F_{1.57,9.4}$ = 1.11, p = 0.35) (Table 3).

Insert Table 3 near here please

Discussion

For the first time, the effect of TYR ingestion on soccer-specific exercise (iSPT) and cognitive function within a warm environment (25°C) was investigated. The main finding of the present study was that a pre-exercise dose of 300 mg·kg body mass^{-1} TYR was associated with improved vigilance, accepting the primary hypothesis. Vigilance HIT responses were significantly increased on average by 9 ± 28% (p = 0.015) with MISS responses significantly decreased on average by 31 ± 29% (p = 0.013) in TYR compared to PLA. This improvement was accompanied by a significant increase of 13 ± 36% (p = 0.039) in RTIME in the TYR condition. This novel finding suggests that ingestion of TYR, a catecholamine precursor, may improve cognitive function during exercise-heat stress and possibly influence the perception of psychological effort. However, TYR ingestion had no effect on physical performance, as the distance covered during iSPT was similar in both conditions, which indeed supports the majority of literature in this area (Strüder et al. 1998; Chinevere et al. 2002; Sutton et al. 2005; Watson et al. 2012; Tumilty et al. 2014).

The present study provides a novel paradigm for the use of TYR in relation to soccer-specific exercise (iSPT), which offers ecological validity and widens the application of the supplement, from previously military (Banderet and Lieberman 1989; Neri et al. 1995; Deijen et al. 1999; Lieberman et al. 2005; Mahoney et al. 2007) and individual sport (Strüder et al. 1998; Chinevere et al. 2002; Sutton et al. 2005; Watson et al. 2012; Tumilty et al. 2014) biased designs. The current findings extend and support the large body of literature demonstrating that TYR is an effective nutritional supplement for alleviating stress-induced deficits in cognitive function (Banderet and Lieberman 1989; Neri et al. 1995; Deijen et al. 1999; Lieberman et al. 2005; Mahoney et al. 2007). During periods of stress, there is a marked decrease in the synthesis of central catecholamine neurotransmitters at the point of exhaustion, inducing partial depletion of catecholamine concentration occurring in the hippocampus and striatum as demonstrated in rodents (Bailey et al. 1993; Meeusen et al. 1997). The proposed mechanism for the provision of supplementary TYR (DA and NA precursor) is to increase central catecholamine neurotransmission, which appears to be advantageous during stressful situations by maintaining facets of cognitive function. It has previously been shown that TYR improves the behavioural response to heat-stress and increases central NA release, albeit in rodents and not humans (Lieberman et al. 2005). The present novel data supports this rodent data (Lieberman et al. 2005), with

the observed improvement in vigilance in the TYR condition during exposure to exercise-heat stress (Fig. 1). However, mechanistic cause and effect data to support this proposed mechanism is not provided from the employed experimental design, as plasma concentrations of TYR, LNAA or catecholamines were not measured.

In the present study TYR improved subjects vigilance, compared to a placebo, as a main effect (increased HIT and decreased MISS responses, see Table 1 for response descriptions and Fig. 6 and Table 2 for data). This improvement was evident across all cognitive test time points, even prior to the commencement of iSPT. Thus, on average, TYR supplementation may be beneficial to soccer players throughout match play in warm environments, rather than specifically during the latter stages, when fatigue is suggested to occur (Meeusen et al. 2006a). This finding was coupled with a significant increase in RTIME, implying that subjects felt more psychologically ready after the bouts of exercise-heat stress in TYR. This novel soccer-specific data, suggests that TYR may augment mental alertness during periods of stress and as a result, contribute to an increase in cognitive performance. Similar paradigms are seen in military focused research with army personnel reporting 'clearer thinking' and a decrease in adverse moods associated with extreme environmental stress (cold and hypoxia) after TYR supplementation, which coincided with a reduction in cognitive performance impairments (Banderet and Lieberman 1989). Despite the improvement in vigilance, there was no significant difference in the dual-task cognitive test scores between conditions in the present study. Lack of statistical significance within the dual-task may derive from the high inter-individual variation in performance (e.g. individuals with very good or poor dual-task skills) of the task, decreasing the chance of observing statistically significant differences between treatment groups, as identified by Hope et al. (1998).

Despite the sound theoretical basis for the use of TYR, the current study failed to demonstrate any improvement in physical performance after TYR ingestion, showing no change in the distance covered during iSPT between conditions (Fig. 5). Furthermore, no effect of time was observed with subjects covering a similar distance in each half of the protocol, highlighting an absence of fatigue. Fatigue is expected after such high intensity exercise in warm conditions, thus this finding is surprising and may have limited the likelihood of TYR exerting any beneficial effect on physical performance. However, this novel finding, specific to team sport performance (iSPT), is concurrent with several other studies in which no beneficial effect of TYR was observed on endurance performance (Strüder et al. 1998;

Chinevere et al. 2002; Sutton et al. 2005) in temperate conditions, or exercise to exhaustion (Watson et al. 2012) and a self-paced time-trial (Tumilty et al. 2014) in hot environments. Conversely, two previous studies from the same authors have demonstrated that the availability of TYR influences the capacity to perform exercise capacity in the heat during constant-load cycling (Tumilty et al. 2011, 2013). Tumilty et al. (2011) observed a 15 ± 11 % increase in exercise capacity during a cycling trial in the heat (30 °C). This is the only study to date, to show a physical benefit of TYR ingestion, despite the efforts of Watson et al. (2012) with a comparable exercise protocol, dosage and rise in circulating TYR to Tumilty et al. (2011). Additionally, Tumilty et al. (2013) confirmed that ingesting a TYR/phenylalanine-free amino acid mixture (to deplete blood TYR levels) reduces exercise capacity in the heat compared to a balanced amino acid mixture (containing TYR), which supports the role of TYR availability in exercise-induced fatigue in the heat. However, as the majority of literature (Strüder et al. 1998; Chinevere et al. 2002; Sutton et al. 2005; Watson et al. 2012; Tumilty et al. 2014), including the current work, contradicts this recent evidence (Tumilty et al. 2011, 2013), it appears that acute ingestion of 150-300 mg·kg body mass^{-1} TYR does not provide an ergogenic effect on a plethora of exercise modalities performed in hot, warm and temperate conditions.

It is not completely clear why there are opposing physical performance findings in the aforementioned studies (Strüder et al. 1998; Chinevere et al. 2002; Sutton et al. 2005; Tumilty et al. 2011; Watson et al. 2012; Tumilty et al. 2014). One possible reason may be the differences in subject's aerobic fitness, training status and experience with exercise testing between studies as this may influence the effects of TYR on performance, however this is merely speculation. Furthermore, Tumilty et al. (2014) suggest that the magnitude of activation of the catecholamine system, subsequent to the stress induced by the different exercise protocols may provide a possible explanation. Under conditions which are not highly stressful, cerebral levels of tyrosine hydroxylase are saturated with substrate, thus the use of supplementary TYR should not significantly increase central catecholamine synthesis or improve exercise tolerance or performance (Lehnert et al. 1984; Foley and Fleshner 2008). Hence it is not surprising that previous studies investigating the effects of TYR in temperate conditions (Strüder et al. 1998; Chinevere et al. 2002; Sutton et al. 2005) failed to observe an ergogenic effect. By utilising the iSPT protocol in the present study, we attempted to combine intense physical exertion with elevated ambient temperature (25 °C) to create a sufficiently demanding environment to alter central catecholamine neurotransmission. However, the protocol did not produce a sufficiently stressful

environment as anticipated, recording very similar end HR values (175 and 177 b·min^{-1} in TYR and PLA, respectively) to Watson et al. (2012) (177 and 175 b·min^{-1} in TYR and PLA, respectively) and Tumilty et al. (2011) (174 and 177 b·min^{-1} in TYR and PLA, respectively). As iSPT is an individualised, valid and reliable protocol with regard to internal and external load of soccer, increasing the intensity *per se* of the protocol is precluded for such reasons. However, increasing the ambient temperature (from 25 °C to >30 °C) that iSPT is performed within could likely manifest a sufficient level of stress to up-regulate catecholamine turnover. This may also induce higher T_{re} values and a so-called 'critical' internal temperature (38- >40 °C) (Cheung and Sleivert 2004), which is suggested to coincide with exhaustion during prolonged exercise in the heat (Nielsen et al. 1993; González-Alonso et al. 1999).

The methodology of the present study contains several limitations, which should be considered in future research. As previously mentioned, the present study did not assess plasma concentration/ratio of TYR and LNAA, which limits cause and effect relationships. However, as previous studies observed significant elevations in plasma/serum TYR after administering 150 mg·kg body mass^{-1} (Tumilty et al. 2011; Watson et al. 2012), it is assumed that a similar, if not greater rise may have occurred in the present study after ingestion of a double-dose (300 mg·kg body mass^{-1} TYR in total). An investigation into the pharmacokinetics of TYR levels in the blood, using a variety of doses, would perhaps provide further elucidation and a basis for future exercise studies. Moreover, the TYR supplement administered in the present study was sourced from an online sport nutrition company, the same company used by Tumilty et al. (2011). This issue was highlighted by Watson et al. (2012), due to the known uncertainty relating to the composition of some widely available nutritional supplements. Although this is important to consider, the TYR supplement used in the present study was analysed via HPLC to assess its purity, which was found to be satisfactory (>90%). However, we highly recommend that future research utilise supplements from medical nutrition companies to minimise risk of contamination, in line with Watson et al. (2012). Furthermore, the timing of the cognitive assessments (PRE, HT, EOHT and POST) may also be considered a limitation. There is evidence to suggest that cognitive function may be impaired or disturbed during maximal exercise (McMorris and Keen 1994), with a rapid return to baseline after cessation of exercise (Dietrich and Sparling 2004). Such disturbances may be due to a larger cerebral emphasis on motor outputs during exercise, at the expense of the cognitive tasks (Dietrich and Sparling 2004). Therefore, future work should aim to evaluate cognitive function during

the employed exercise protocol. Finally, the cognitive assessments were all completed post-ingestion of the supplement, which does not allow for a pre-post ingestion comparison to be made. Although this may have improved the study design, the additional repetition of the cognitive tests may have become tedious and consequently decrease the engagement of the subjects.

As soccer is highly dependent upon the execution of motor skills and decisional-based tasks, minor decrements in cognitive performance could significantly alter the outcome of a game (Meeusen et al. 2006a). Since many important soccer matches and tournaments are played in hot climates (>25 °C), including the Champions League and World Cup finals, ingestion of TYR as a pre-game supplement may enhance the decision-making capabilities of soccer players. Additionally, on-pitch referees may also benefit from TYR supplementation, as previous research has shown that elite referees cover a similar distance to players during a game (Weston et al. 2011), thus similar internal and external loads are experienced by referees [iSPT replicates these loads in an individualised, valid and reliable manner (Aldous et al. 2013)]. The use of the newly validated iSPT (Aldous et al. 2013) protocol to replicate individualised internal and external soccer-specific loads, provides novel data regarding TYR supplementation within team sport based exercise. Furthermore, as previous military research exclusively explores cognition and mood state with and without TYR supplementation within cold and/or hypoxic conditions (Banderet and Lieberman 1989; Mahoney et al. 2007; O'Brien et al. 2007), the present findings may provide a stimulus for exploration within hot environments, as army personnel may undergo similar exercise-heat-stress situations during training and in combat. The current study replicated the maximum dose (300 mg·kg body mass^{-1} TYR) previously administered in the literature (Mahoney et al. 2007; O'Brien et al. 2007) in a drink form, without any adverse side effects, which may be useful for future research investigating large doses of TYR.

Conclusions

In summary, this study demonstrated for the first time, that ingestion of 300 mg·kg body mass^{-1} TYR significantly improved vigilance and RTIME, but not physical performance, when exposed to individualised soccer-specific exercise (iSPT) in a warm environment. This suggests that TYR availability is associated with improvements in aspects of cognitive performance when exposed to

acute stress and therefore may be beneficial as a nutritional supplement prior to soccer match-play in hot conditions. The exact mechanism to explain these findings is at present unclear and although previous literature (Lehnert et al. 1984; Lieberman et al. 2005; O'Brien et al. 2007) may provide reasonable speculation, these concepts must be explored further before definite conclusions can be made. Future research should investigate the pharmacokinetics of TYR and also assess the effects of chronic supplementation on health and performance.

Conflicts of Interest: None

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Table Captions

Table 1 Descriptions of each cognitive test response (HIT, MISS, FALSE and TRACKING) for vigilance and dual-task assessments

Table 2 Vigilance scores in TYR and PLA conditions for all time-points measured. Overall main effect observed for HIT and MISS scores in TYR condition. Values are mean ± SD

Table 3 Dual-task cognitive test scores in TYR and PLA conditions. Values are mean ± SD

Figure Captions

Fig. 1 Group mean heart rate (HR) ($b.min^{-1}$) responses across both TYR and PLA conditions. Values are mean ± SD. Participants experienced similar increases in HR during both conditions that were not significantly different. #Denotes significant differences over the 90 min protocol ($p < 0.05$)

Fig. 2 Group mean-weighted skin temperature (**a**) and mean core temperature (**b**) responses to exercise across both TYR and PLA conditions. Values are mean ± SD. Participants experienced a similar rise in core and skin temperature during both conditions that was not significantly different. #Denotes significant differences over the 90 min protocol ($p < 0.05$)

Fig. 3 Group mean thermal sensation (TSS) (**a**) and rating of perceived exertion (RPE) (**b**) responses to exercise across both TYR and PLA conditions. Values are mean ± SD. Participants experienced a similar rise in both TSS and RPE during both conditions that was not significantly different. #Denotes significant differences over the 90 min protocol ($p < 0.05$)

Fig. 4 Group mean readiness to invest physical effort (RTIPE) (**a**) and readiness to invest mental effort (RTIME) (**b**) across both TYR and PLA conditions. Values are mean ± SD. Note: HT = half-time and EOHT = end of half-time. *Denotes significant difference between conditions ($p < 0.05$). #Denotes significant differences over time ($p < 0.05$)

Fig. 5 Group mean distance covered (m) during the first half (FH), second half (SH) and total distance (TD) covered during iSPT across both TYR and PLA conditions. Values are mean ± SD. Participants covered a similar distance during both conditions that was not significantly different

Fig. 6 Group mean vigilance cognitive test responses (HIT, MISS and FALSE) across both TYR and PLA conditions. Values are mean ± SD. *Denotes significant difference between conditions ($p < 0.05$)

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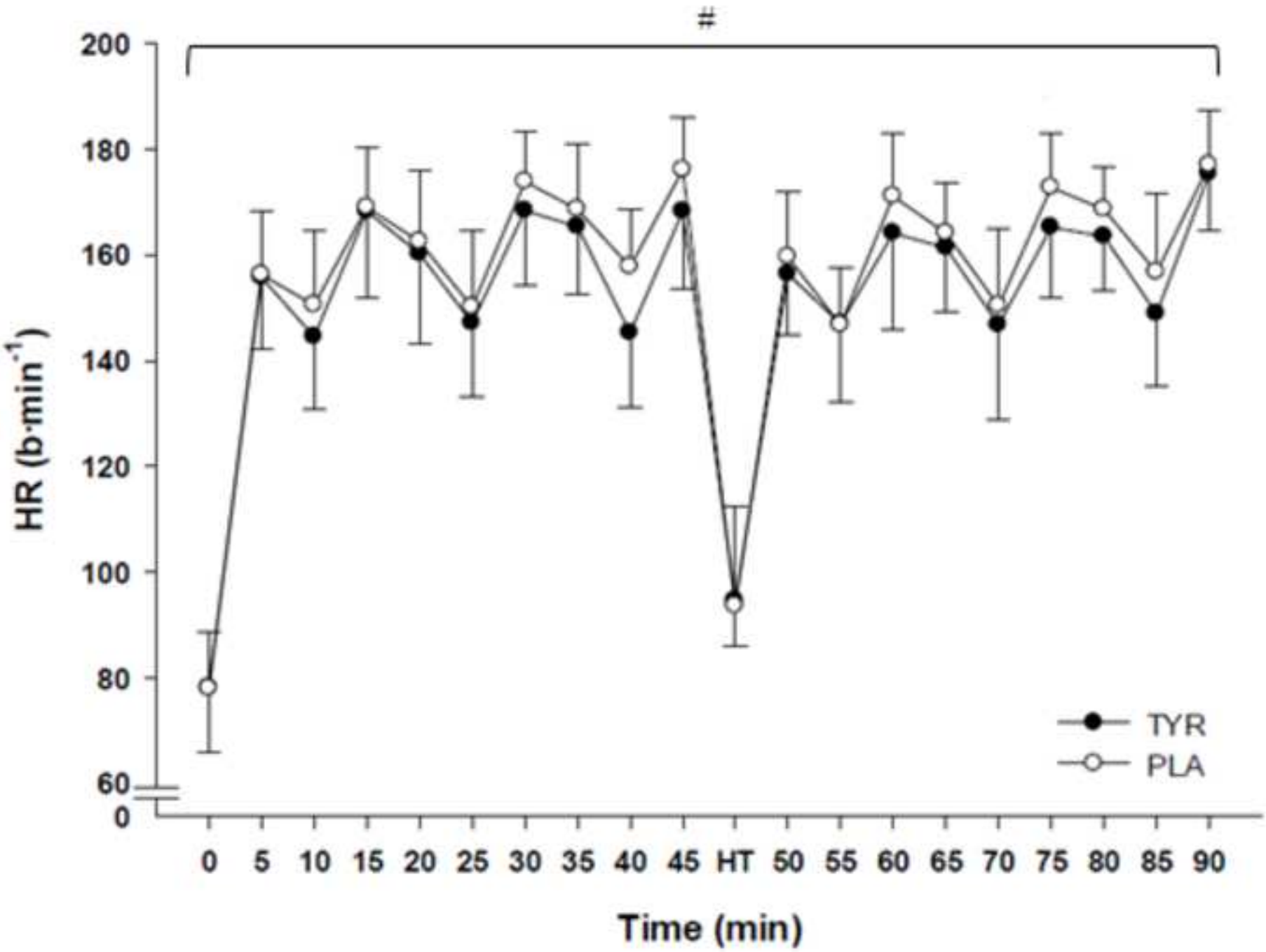

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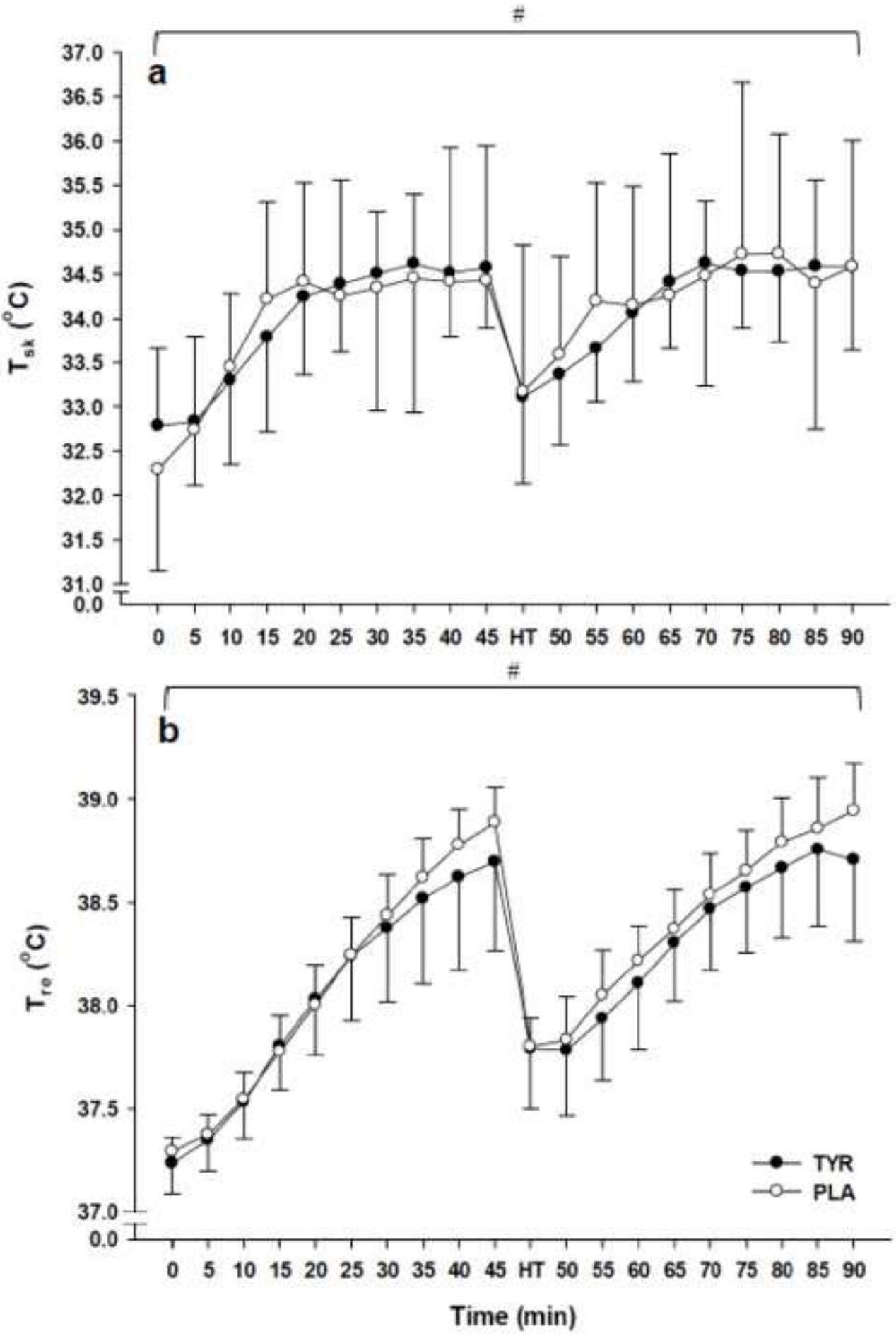

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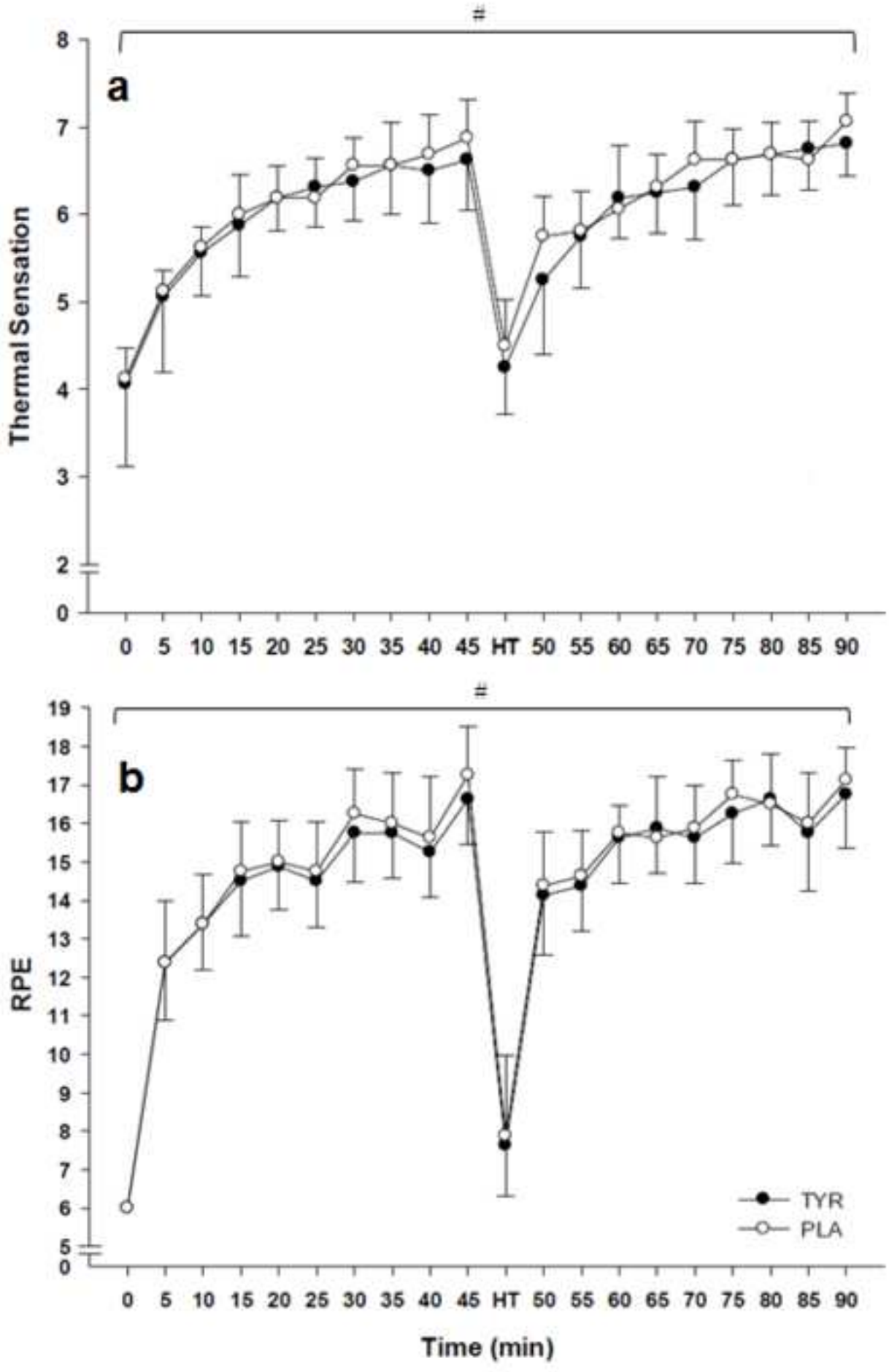

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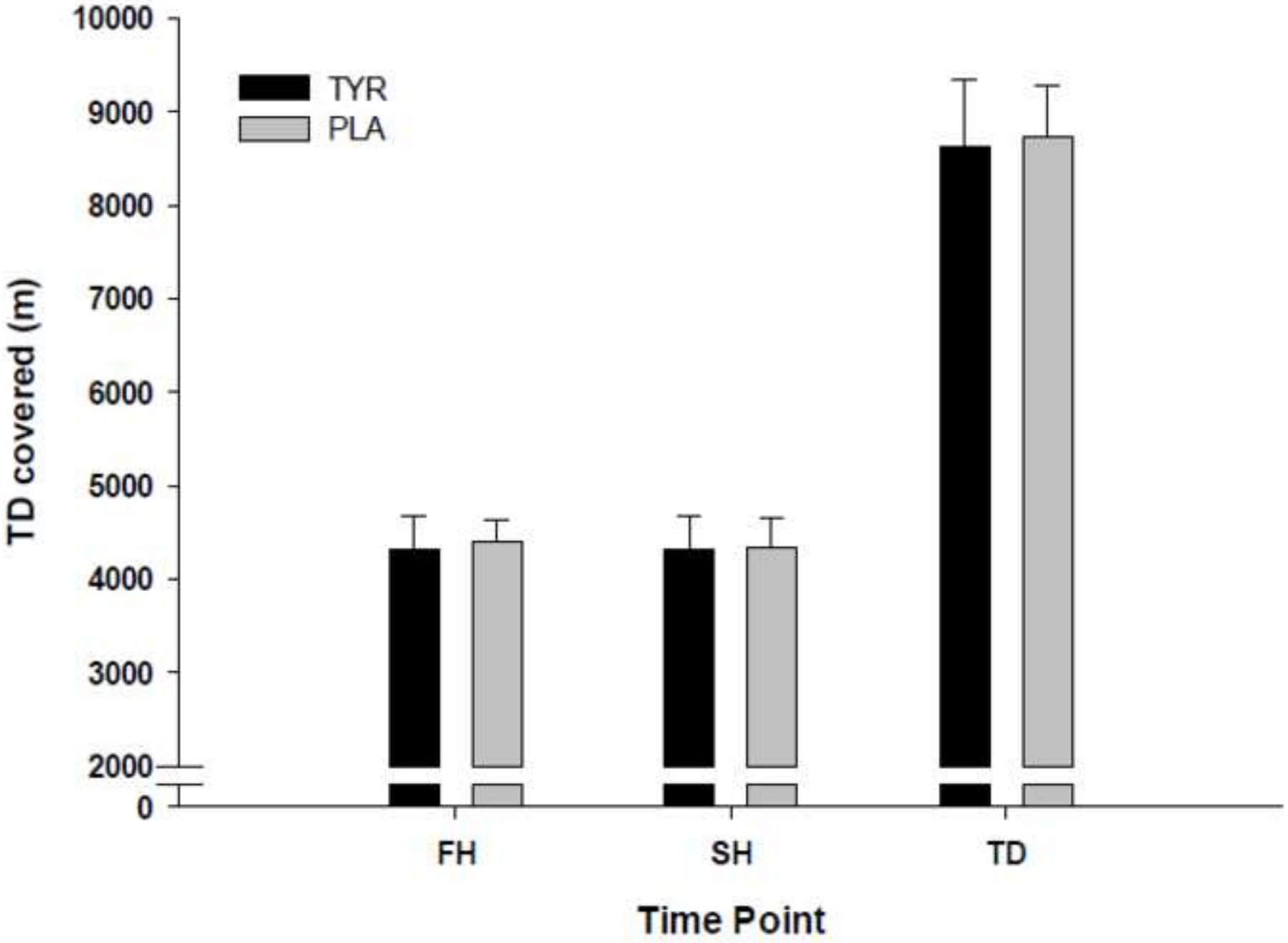

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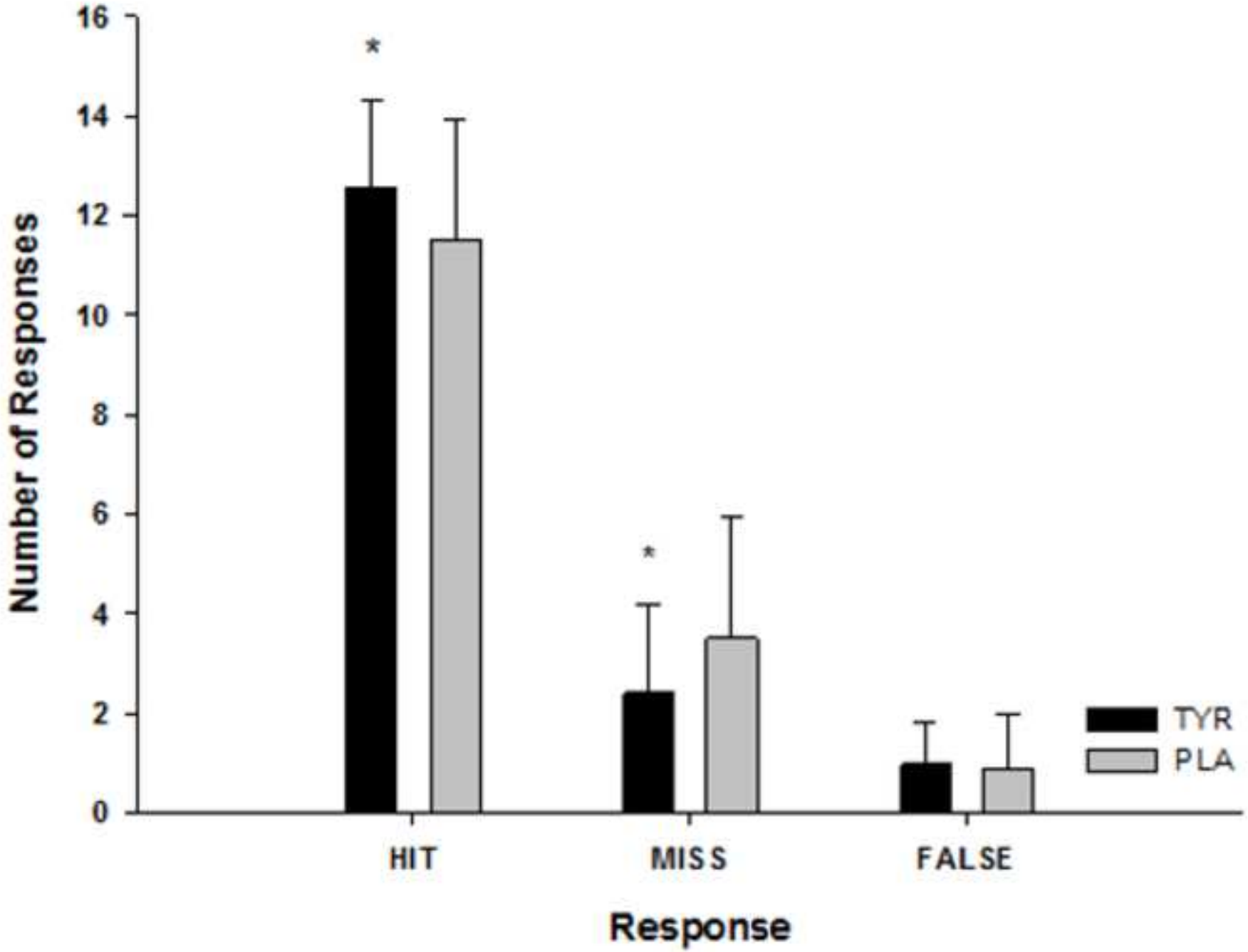

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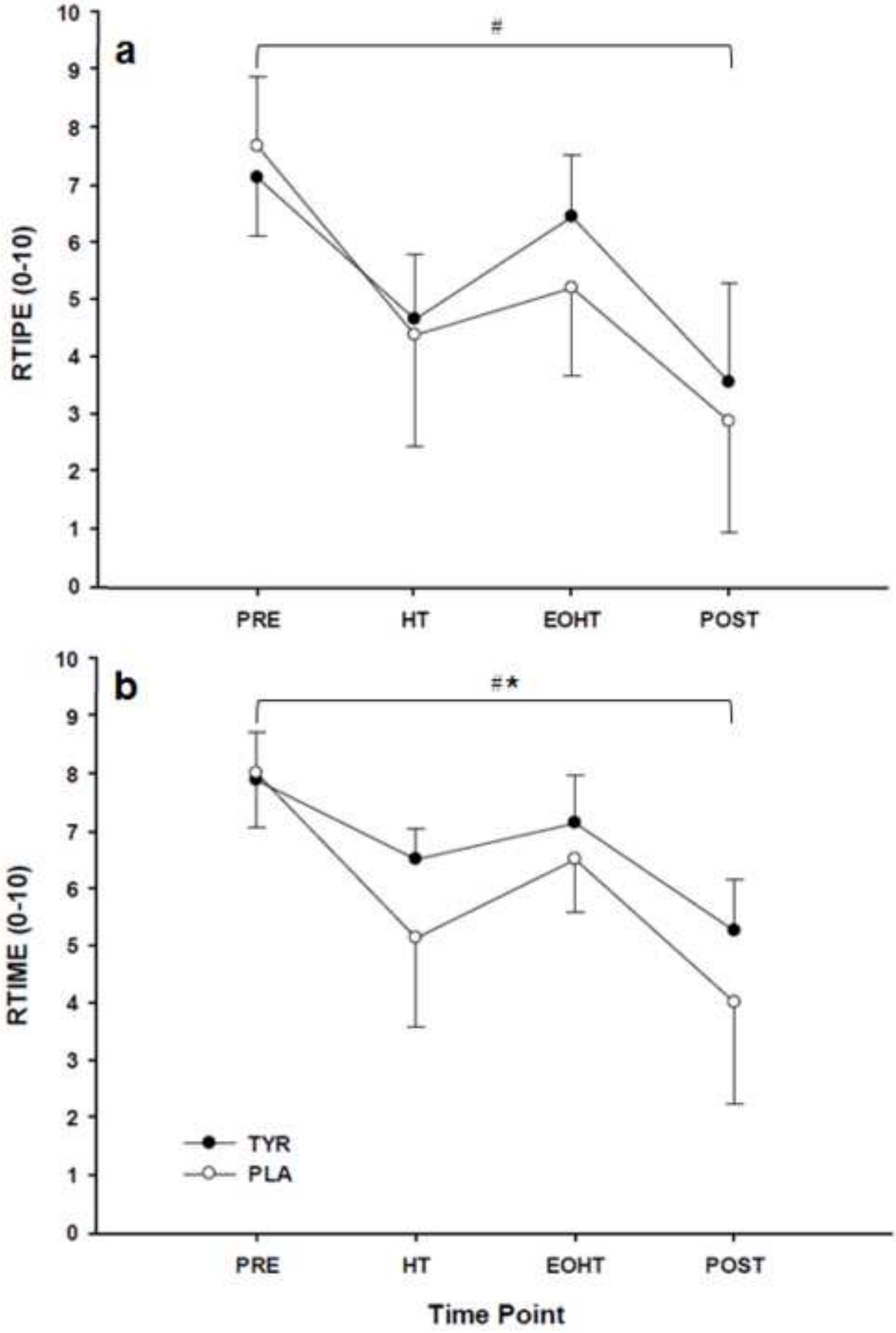

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Response	Vigilance	Dual-task
HIT	Correctly identifying a duplicate number	N/A
MISS	Failing to identify a duplicate number	Failing to identify a present icon
FALSE	Incorrectly identifying a duplicate number	Incorrectly identifying an icon is present when it is not
TRACKING	N/A	Ability to track a moving target (%)

Table
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Response	Treatment	Score			
		0 min	HT	EOHT	90 min
HIT	TYR	12.8 ± 2.2	12.6 ± 1.8	12.4 ± 1.4	12.5 ± 1.8
	PLA	11.8 ± 2.1	11.4 ± 2.8	11.5 ± 2.5	11.4 ± 2.8
MISS	TYR	2.1 ± 2.2	2.4 ± 1.8	2.6 ± 1.4	2.5 ± 1.8
	PLA	3.3 ± 2.1	3.8 ± 2.7	3.4 ± 2.6	3.6 ± 2.8
FALSE	TYR	1.2 ± 0.6	0.9 ± 0.6	1 ± 1.3	0.9 ± 0.8
	PLA	0.5 ± 0.8	1.1 ± 0.8	0.8 ± 0.9	1.1 ± 1.7

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Response	Treatment	Score
Dual-task TRACKING (%)	TYR	72.3 ± 6.7
	PLA	70.3 ± 6.3
Dual-task MISS	TYR	1.1 ± 0.3
	PLA	1.1 ± 0.5
Dual-task FALSE	TYR	0.3 ± 0.8
	PLA	0.5 ± 0.7